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Letter to the editor: 'Human based systems: Mechanistic NASH modelling just around the corner?'

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We congratulate Boeckmans et al., on their comprehensive review of current models of non-alcoholic fatty liver disease (NAFLD) presented 'Human based systems: Mechanistic NASH modelling just around the corner?' (1). NAFLD affects a large number of people worldwide and despite advances in our knowledge of the condition, further research is needed to identify biomarkers and target molecules for therapy. We agree that results from animal experiments may not always represent human pathophysiology accurately and we believe that relevant human *in vitro* models can be informative in understanding NAFLD disease mechanisms and target therapies. In addition, such models should help rationalisation of animal experiments in line with the 3R principles of replacement, reduction and refinement.

We have developed a model of metabolic nutrient overload using the human hepatocyte HepG2/C3A cell line and have shown that treatment of these cells with energy substrates; lactate, pyruvate, octanoate and ammonia (LPON) recapitulates the sequence of hepatocellular events in dietary-induced NAFLD, including cytoplasmic fat accumulation, enhanced cellular respiration and increased production of reactive oxygen species leading to mitochondrial dysfunction [2, 3]. Furthermore, we have shown that media from fat-loaded C3A cells can activate stellate cells *in vitro* and thus represent a valid model for studying NAFLD pathophysiology (Figure 1, Supplementary data). The disadvantage, however, of C3A cells is the low expression of hepatic Cytochrome P450 (CYP) enzymes, while other key mediators of intermediary metabolism, such as the urea cycle-related enzymes, are also expressed at low levels.

We feel that the value of the human hepatoma HepaRG cell line [4] as a putative *in vitro* disease model for NAFLD has perhaps been understated in the review (1), with HepaRG cells differing significantly to other hepatic cell lines mentioned:

- (i) HepaRG cells are originally derived from a female with a grade I well-differentiated hepatocellular carcinoma concurrent with hepatitis C and present under standard culture conditions as a 50:50 co-culture of hepatocyte and biliary epithelial cholangiocyte-like cells;
- (ii) HepaRG cells are highly-differentiated and express CYP enzymes and constitutive hepatic nuclear receptors at levels comparable to freshly-isolated primary human hepatocytes, without the need for inducers and also display other differentiated functions including intact ammonia metabolism and urea production pathways and functional bile canaliculi formation;
- (iii) HepaRG cells display a stable genotype and maintain phenotypically-differentiated functions in culture for at least four weeks, thus making them ideal cells for studying the pathophysiology of NAFLD [4, 5].

We have found HepaRG cells to be preferable to C3A cells within our nutrient overload model of NAFLD, and have shown that incubation of these cells with LPON or oleate induces cytoplasmic fat accumulation similar to that in C3A cells and in dietary-induced NAFLD (Figure 2, Supplementary

data). Co-culturing HepaRG cells with key pathophysiological players in liver disease such as mesenchymal stem cells, Kupffer and stellate cells could further optimise an *in vitro* model of NAFLD.

Finally, utilising a NAFLD *in vitro* model in tandem with novel techniques, such as Electric Cell Substrate Impedance Sensing (ECIS), would permit, non-invasive, real-time study of cellular integrity and adhesion in fat-loaded cells and facilitate high throughput screening of novel compounds of therapeutic interest for NAFLD.

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Conflict of interest:

The authors have no conflict of interest to declare with respect to this manuscript.

Authors' contributions:

KM drafted the letter to the editor. FV, KS, SM PH and JP edited the letter. All authors reviewed and approved the final version.

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Letter to the editor: Supplementary Data Figure 1:

‘Human based systems: Mechanistic NASH modelling just around the corner?’

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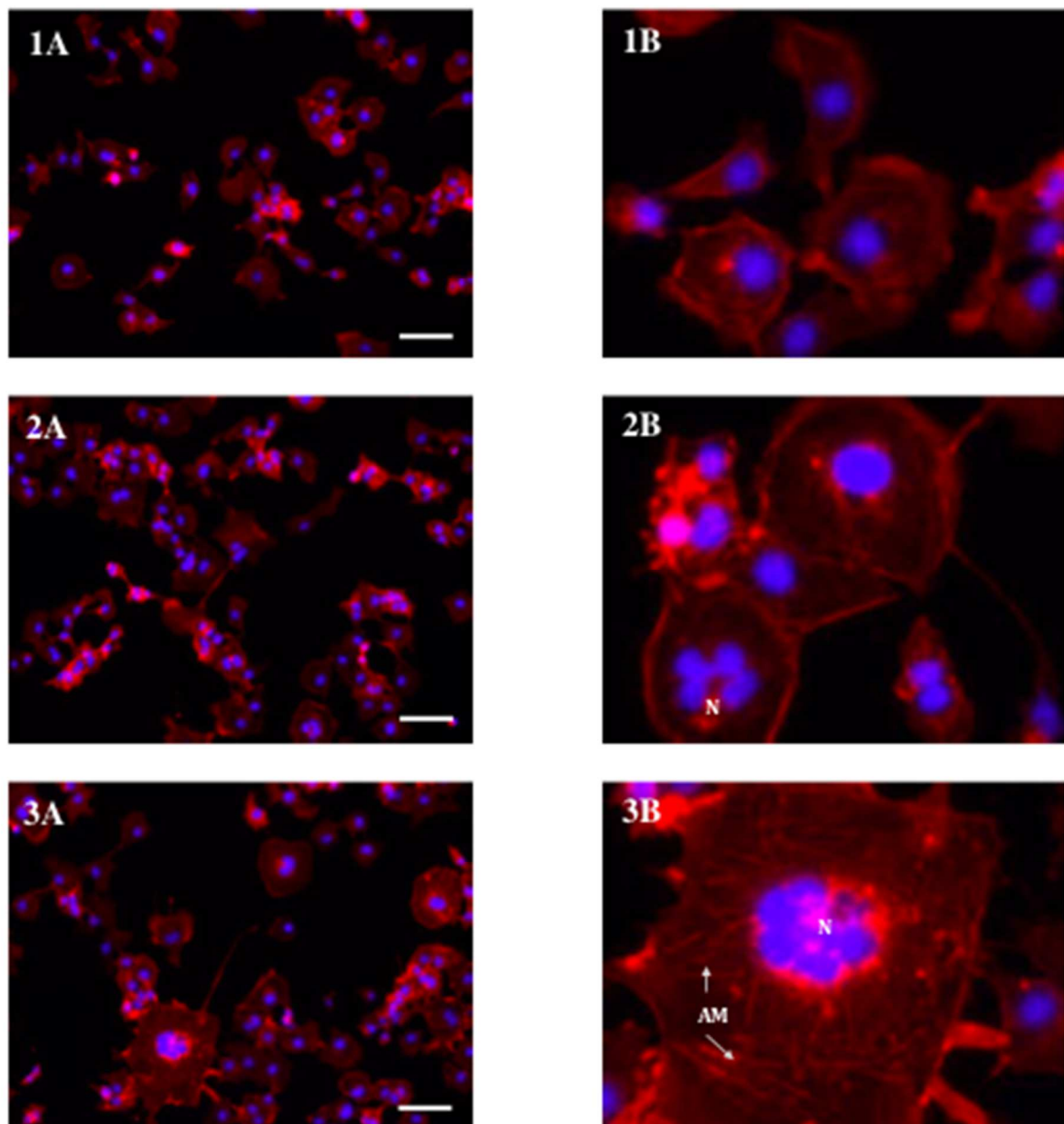


Figure 1: LX2 stellate cells exposed to supernatant from, LPON (2A&B) or oleate (3A&B) - treated C3A cells showed enlargement and proliferation compared to untreated controls (1A&B). Phalloidin staining showed increase in cellular actin microfilaments (AM arrows).

Letter to the editor: Supplementary Data Figure 2:

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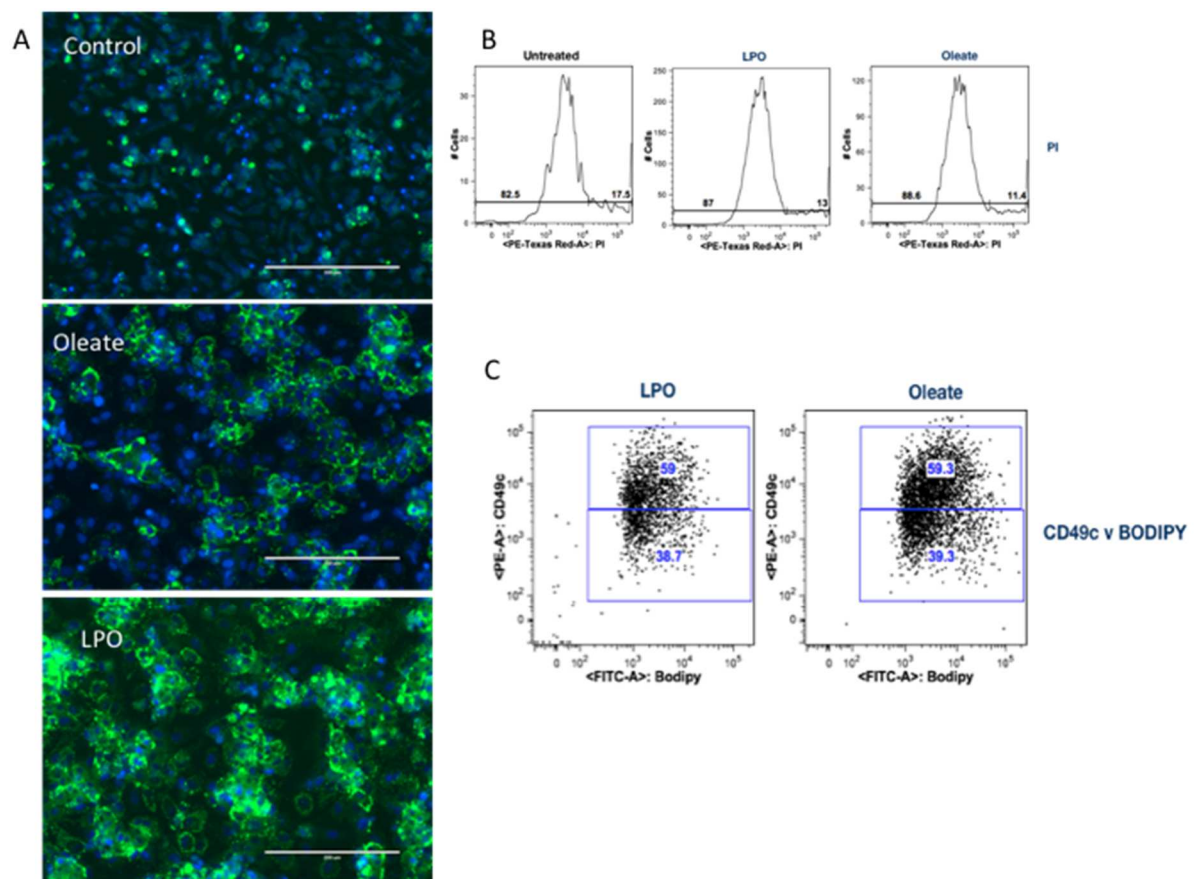


Figure 2: A: Day 9 HepaRG cells treated with either oleate or lactate, pyruvate and octanoate (LPO) and stained with bodipy and hoescht. There is localised uptake of fat following both oleate and LPO treatments, with more cytoplasmic lipid staining following LPO treatment. B: Flow cytometry analysis of Day 10 HepaRG cells stained with PI showing no difference in level of viability between conditions. C: Flow cytometry of day 10 HepaRG cells staining for bodipy and CD49c showing that both hepatocytes, CD49c positive, and cholangiocytes have taken up both oleate and LPO.